Subterranean Biology 10: 1-16, 2012 (2013)
doi: 10.3897/subtbiol.10.4758

Origin of the interstitial isopod *Microcharon* (Crustacea, Microparasellidae) from the western Languedoc and the northern Pyrenees (France) with the description of two new species

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ABSTRACT

The interstitial groundwater genus *Microcharon* (Crustacea, Isopoda, Microparasellidae) is highly diversified in southern France. A new species, *Microcharon boulanouari* n. sp. is described from the Aude River, whereas specimens from the Lachein River in the central Pyrenees are reassigned to another species, *M. ariegensis* new to Science. *Microcharon boulanouari* n. sp. is closely related to the species of the group *rouchi* and may belong to the phylogenetic western Mediterranean lineage. The two-step model of colonization and evolution provides an understanding of the origin and age of this stygobite. *Microcharon boulanouari* n. sp. is derived from marine ancestors that lived in the interstitial littoral shallow bottoms of the Atlantic embayment which covered southwestern France at the very beginning of the early Eocene period. Both the regression of this gulf at the start of the Eocene and the Pyrenees uplift may have played a major role in the evolutionary history through vicariance of *Microcharon boulanouari* n. sp. and of the northern Pyrenean species of the group *rouchi*.

Key words: ground water, interstitial isopods, *Microcharon,* systematics, vicariance, western Languedoc, northern Pyrenees, palaeobiogeography

INTRODUCTION

Species of the minute crustacean isopod *Microcharon* (Microparasellidae) live in the fresh ground waters of river and aquifer sediments as well as in the interstitial water of marine sandy beaches and shallow bottom beaches (Lévi 1951, Spooner 1959, Bocquet 1970, Coineau 1986). Their ability to disperse is very feeble since they are holobenthic and exhibit no swimming larvae. Nevertheless, the genus *Microcharon* Karaman, 1933 has a worldwide distribution, from Polynesia and New Caledonia to Europe, the Mediterranean, the Caribbean and the Galapagos (Delamare Deboutteville 1960, Coineau 1986, Stock 1977, Galassi 1991, Galassi et al 1995a, b). More than 70 species are known from the Mediterranean basin alone both in the northern and the southern areas (Argano & Pesce 1979, Pesce & Galassi 1988a, b, 1990, Pesce & Tetè 1987, Galassi 1991, Coineau 1994, Galassi et al 1995a, b, Boulanouar et al 1995, Yacoubi Khebiza et al 1997, 1999, Stoch & Galassi 2002).

Inland species are derived from marine ancestors left in fresh ground waters by Tethys regressions (Delamare Deboutteville 1960, Coineau 1971, 1994, Stock 1977, Boutin 1993, Wägele 1990, Galassi 1991, Galassi et al 1995b, Coineau et al 2001, Stoch & Galassi 2002). Previous works on phylogeny and biogeography of inland species have shown that species with more characters in an apomorphic state resulted from recent regressions whereas species exhibiting more plesiomorphic character states were left by ancient marine withdrawal during the late Cretaceous period (Coineau 1994, Coineau et al 1994, Galassi et al 1995b, Stoch & Galassi 2002). Vicariance evolution may have worked at different scales of space and time: at the scale of the Mediterranean, plate tectonics and large transgressions resulted in different lineages. At the regional and local scales, regressions and orogenesis were responsible for the differentiation of sister species (Coineau 1994).

The genus *Microcharon* is widespread in the southern ground waters of France (Ferreira et al 2003). Several species are located within the subterranean waters of the hydrographic system of the Rhône River (Coineau 1968, 1986, Dole & Coineau 1987). In southwestern France, only two species are known to occur. *Microcharon boui* Coineau, 1968 occurs in wells at Albi in the southern Massif central and *M. rouchi* Coineau, 1968 inhabits the ground waters of the Garonne and the Adour River hydrographic systems in the northern Pyrenees. Finally, *M. angelieri* Coineau, 1963 occurs in the phreatic waters of the Tech River in the Roussillon.

Received 5 February 2012; Accepted 11 June 2012

Therefore, the genus *Microcharon* is diversified in southern France. Different species are endemic to the various aquifer basins.

In the Roussillon and the western Languedoc regions (Fig. 1), four species new to Science have been discovered in the framework of the European PASCALIS Project in 2003 (Coineau et al 2005, Artheau 2006). One of these species from the ground waters of the Aude River is described hereafter. Moreover, the purpose of this paper is to provide an understanding of the origin of the representatives of the genus *Microcharon* from the western Languedoc and the northern Pyrenees within a distributional pattern and a palaeogeographic context.

MATERIAL AND METHODS

Sampling of specimens: All specimens were collected in wells using a Cvetkov net:

- More than 100 individuals with few males, many females and post-embryonic stages, from well ROU A37/D4 (X 456190- Y 47892810) at Trèbes, near the Aude River, August 5th 2003;
- 1 male, 11 females, 3 post-embryonic stages, from well ROU 139/D4 (X 436680 - Y 4767990) at Limoux, June 26th 2003;
- 1 male, from well ROU 138/D4-PN39 (X 436500 - Y 4754050) at Espéraza, June 26th 2003;
- 15 males, 11 females (with 1 ovigerous specimen), from well ROU 142/D4 (X 450190 -Y 4784380), at Périac-Carcassonne, August 4th 2003;
- 1 young male, 4 females, from well ROU 133/D4 (X 452870 - Y 4784460), Barette garden at Carcassonne, August 4th 2003;
- 4 females, 1 post-embryonic stage, from well ROU 143/D4 (X 483670 -Y4786830), at Ornaisons, July 26th 2003.

Study of the new species

Several males and females have been completely dissected and mounted on slides in polyvinyl-lactophenol. Five specimens of the syntype series (individuals of Trèbes site) have been deposited at the Museum National d'Histoire Naturelle, Paris: MNHN-Is6302. Other specimens in authors' collections. Furthermore, the diversity of specimens of *Microcharon rouchi* from two different areas of the northern Pyrenees is re-examinated.

Historical Biogeography

To assess the origin of the isopods, two types of data have been used. 1. The distributional pattern has been established considering the PASCALIS programme results and the distribution of the Pyrenean species (Coineau 1968). 2. Since freshwater species of *Microcharon* are derived from marine ancestors, palaeogeographic data have been used and a relation between the extension of past embayments and the distribution area has been considered to date the ancestor entrance into the inland subterranean habitat. As for other marine interstitial relicts, the Two Step Model of Colonization and Evolution has been applied. This model is one of the scenarios providing an understanding of the colonization of interstitial inland subterranean waters by marine surface ancestors (Boutin & Coineau 1990, Notenboom 1991, Coineau & Boutin 1992, Holsinger 1994). During the first step, the surface epibenthic marine ancestor entered actively the interstitial sandy biotopes of the littoral shallow bottoms of the sea and evolved in minute interstitial isopods. This phase corresponded to an active dispersion and involved especially progenetic evolutionary processes. The second step involved a progressive and passive settlement of populations of the interstitial seashore crustacean into subterranean freshwater habitats during a regression of the sea. This last phase was described by Stock (1980) as the Regression Model Evolution. Vicariance evolutionary processes occurred as soon as the gene flow was interrupted between the new limnostygobiont and the marine populations remaining on the seashore. At the end of a regression period, a series of speciation events working successively, from the same marine ancestor up to the limnostygobionts, at each change of biotope and at each step of the colonization, resulted in several closely related species. When established in inland fresh waters, the new thalassoid populations continued to evolve mainly through vicariance, due to eventual geologic events acting as barriers responsible for divergences due to isolation of catchments and their stygobiontic populations within the distribution area.

Both palaeogeographical and paleontological results about the study region occupied now by the stygobionts have provided useful data.

RESULTS

Microcharon boulanouari n. sp.

Diagnosis *of Microcharon boulanouari* n. sp.: body segments stocky; distal plumose seta of antennule second article overreaching middle part of article 4; articles 5 and 6 of second antenna narrow and very long; pleotelson long with distal margin concave laterally, strongly convex in median part; female pleopod 2 long with lateral margins curved and distal margin straight; sympod of male pleopod 2 with inner distal corner slightly extended; exopod of third pleopod smooth; sympod of uropod slightly shorter than endopod.

Etymology.

The new species is dedicated to Dr M Boulanouar, Professor at the Ecole Normale Supérieure and at the University of Marrakesh for his important contribution to the knowledge of the different groups of groundwater isopods from Morocco.

Body length (excluding uropods): 1.60 -1.87 mm in males, 1.68 -1.90 mm in females. Body stocky. All segments of the body are wider than long, except the pleotelson.

Antennule (Fig. 2B). Six-segmented; first segment longer than wide, with 3 small setae and one distal plumose seta; second segment longer than wide, bearing 3 short plumose setae, 2 subdistal small setae and the long distal plumose seta overreaching the middle part of segment 4; following segments all together clearly shorter than the first two segments together; one short apical seta on segment 3, 1 distal plumose seta on segment 4; segments 5 and 6 longer than wide, aesthetasc of segment 5 relatively short; segment 6 armed with 3 long setae, 1 long plumose seta and a long aesthetasc; no sexual dimorphism on the appendage.

Antenna (Fig. 2A). Exopodite with two setae; the last two peduncle segments very long; 8 to 10-articulated flagellum with all articles narrow and very long.

Right mandible (Fig. 2C). *Pars incisiva* armed with 4-5 apical teeth and without *lacinia mobilis*; 3 distally-denticulated spines and 4 short setae; *pars molaris* with 3 setae. Palp 3-segmented: segment 1 without armature, long segment 2 with 2 subdistal denticulated spines; segment 3 bearing 3 denticulated spines, decreasing in length from distal to proximal, and 2 rows of microsetulae.

Left mandible (Fig. 2D). Similar to the right counterpart except for *lacinia mobilis* with 4 small teeth and only 2 denticulated spines. Palp similar to the symmetric one.

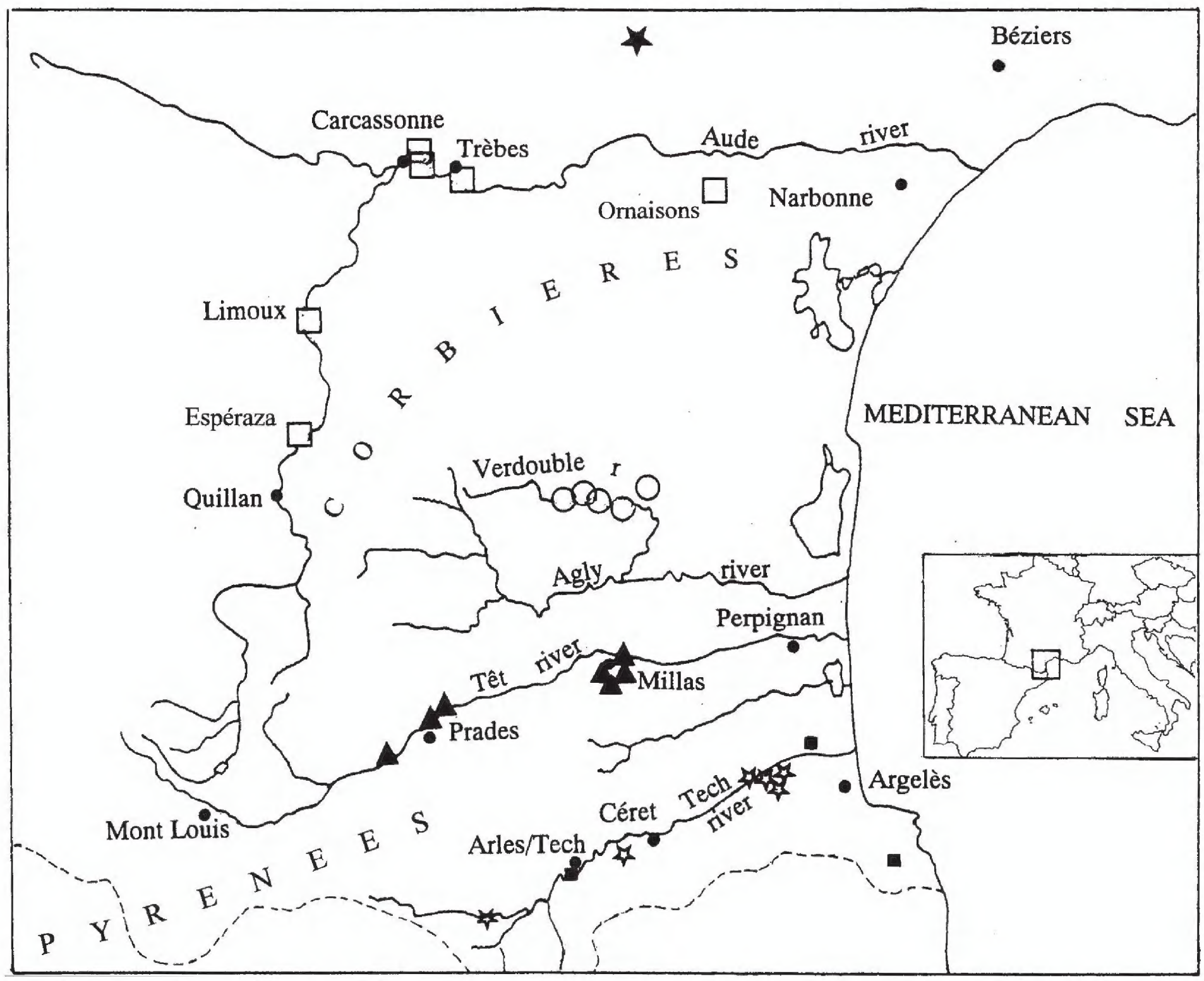

Fig. 1 - Distribution of the inland species of the genus *Microcharon* from the western Languedoc and the Roussillon. *M. boulanouari* n. sp. (open squares) from the Aude River; undescribed species new to science from subterranean waters of the Verdouble River (open circles), the Têt River (filled triangles), and the Tech River (open stars). *M. angelieri* (filled squares).

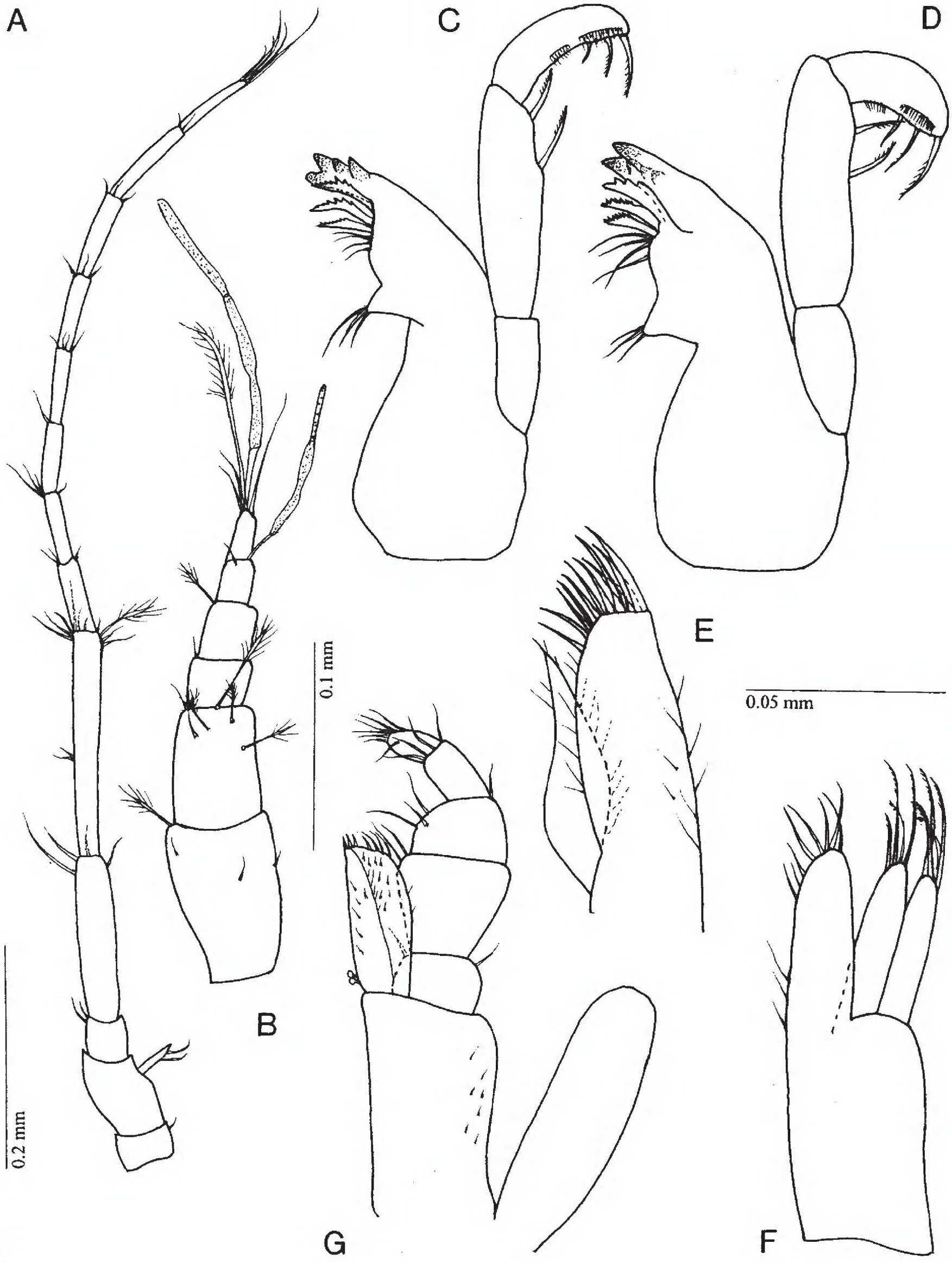

Fig. 2 - *Microcharon boulanouari* n. sp. A, antenna; B, antennule; C, right mandible; D, left mandible; E, maxillule; F, maxilla; G, maxilliped. Scale bars: 0.05 mm: C, D, E, F; 0.1mm: B, G; 0.2 mm: A.

Maxillule (Fig. 2E). Inner lobe narrow and pointed, with 1 distal and 2 subdistal thin setae. Outer lobe armed with 11 distal spines; seven and 8 small setae on inner and outer margins respectively.

Maxilla (Fig. 2F). Median and outer endites with 4 long pectinated setae; inner endite scarcely longer than the other, but wider and with 5 nacked apical and 6 short marginal setae.

Maxilliped (Fig. 2G). Basipodal endite with 7 short apical spines, marginal setae and covered with numerous setulae; two coupling hooks on inner margin; articles 2 and 3 of palp wide; palp armature as in figure; epipodite apex reaching distal limit of palp article 1.

Pereiopods (Fig. 3). Basis moderately enlarged with small differences in armature (see figures); propod long and narrow in P6 and P7; dactylus with unequal claws, longest one not too much elongate.

Pleotelson (Fig. 4A) clearly longer than wide (width/length ratio ≥2/5). Lateral margin almost straight with maximum width attained at proximal one-third of length; distal margin slightly concave laterally, strongly convex and rounded in median part. Chetotaxy as in Fig. 4A, with two subdistal plumose setae on dorsal surface

Male first pleopod (Fig. 4C) relatively long, with long exopods (according to Cvetkov 1968 terminology), separated along distal one-third of appendage length. Fold and hyaline fringe both reaching outer third of distal margin of exopod. Six setae located as in figure of which 2 subdistal setae very short, and 2 distal most longer and subequal.

Male second pleopod (Fig. 4B). Sympod moderately elongated, with outer margin regularly rounded and inner distal corner slightly marked and round; appendix masculina thin with chamfered edge tip and slightly overreaching sympod apex; exopod well developed and broad.

Female second pleopod (Fig. 4D, E) clearly longer than wide, lateral margins dorsally curved and forming a cavity containing pleopods 3 and 4; distal margin more or less straight, without seta.

Third pleopod (Fig. 4F). Endopod subovoid; exopod without setulae, first segment wide, second segment short and wide, reaching distal margin of endopod.

Uropods (Fig. 3E). Sympod scarcely shorter than endopod; the latter very long and narrow, with the normal subdistal and distal setae.

All specimens examined from the six localities exhibit a similar morphology.

Microcharon rouchi and *M. ariegensis* n. sp.

While comparing *Microcharon boulanouari* n. sp. with *M. rouchi* from several valleys of the Pyrenees, it appeared that the specimens from the Lachein River previously assigned to *M. rouchi* (see Coineau 1968) had to be separated from the individuals of the Basque Country and transferred into another new species, *Microcharon ariegensis* n. sp. on the basis of the differences already mentioned in 1968 (Compared to the individuals from the Basque Country, those from the Lachein River have the antennula aesthetascs longer, the plumose seta of antennula segment 2 shorter, the pereiopodal claws shorter, the second article of pleopod III longer). The types are deposited at the Muséum National d'Histoire Naturelle, Paris: one specimen is designated as the lectotype of *Microcharon rouchi* from the Kakouetta River, number MNHN-Is6301. One individual is designated as the holotype of *M. ariegensis* n.sp. from the Lachein River, number MNN-Is6308.

Diagnosis of Microcharon ariegensis n. sp.

Distal plumose seta of antennula second segment very short; one of pereiopodal claws very long; pleotelson wider than body segments with rounded distal and lateral margins; distal part of sympod of male second pleopod wide with rounded outer corner; female pleopod 2 almost rounded and slightly longer than wide; setulae present along lateral margin of exopod of third pleopod.

Only specimens from the ground waters of the Pyrenean Kakouetta, Nive and Laran Rivers, from the French Basque Country, may be referred to *Microcharon rouchi*. *Microcharon ariegensis* n. sp. inhabits the ground waters of the Lachein River (Coineau 1968), and the Ariège and Hers Rivers in the central northern Pyrenees (Dumas 2000).

Remarks

Microcharon boulanouari n. sp. exhibits affinities with the Pyrenean species *M. rouchi* and *M. ariegensis* n. sp., especially the enlarged pleotelson and distal part of the male pleopod 1. However, compared with these two species, *M. boulanouari* n. sp. is longer and clearly more stocky. Lateral margins of the pleotelson are not curved as in *M. ariegensis* n. sp. (Fig. 5), so that this segment is not the widest of the body. The long plumose seta of antenna 1 second segment is very short in *M. rouchi* and *M. ariegensis* n. sp., while aesthetascs are shorter in *M. boulanouari* n. sp. The male first pleopod is clearly different from this appendage in *M. rouchi* and *M. ariegensis* n. sp. In the latter species, the fold and the hyaline fringe reach the median part of the distal margin of the exopod. In *M. boulanouari* n. sp., they reach this margin at a more lateral location; the distal part of the appendage is wider in *M. rouchi* and *M. ariegensis* n. sp. The female pleopod 2 is also very different in *M. boulanouari* n. sp.: it is more or less round in the Pyrenean species whereas it is clearly longer than wide in *M. boulanouari* n. sp. The length of exopod and endopod of the third pleopod is almost equal in *M. boulanouari* n. sp. vs endopod longer than exopod in the two other species. Furthermore, the outer margin of the exopod exhibits a row of microsetulae in *M. rouchi* and *M. ariegensis* n. sp.

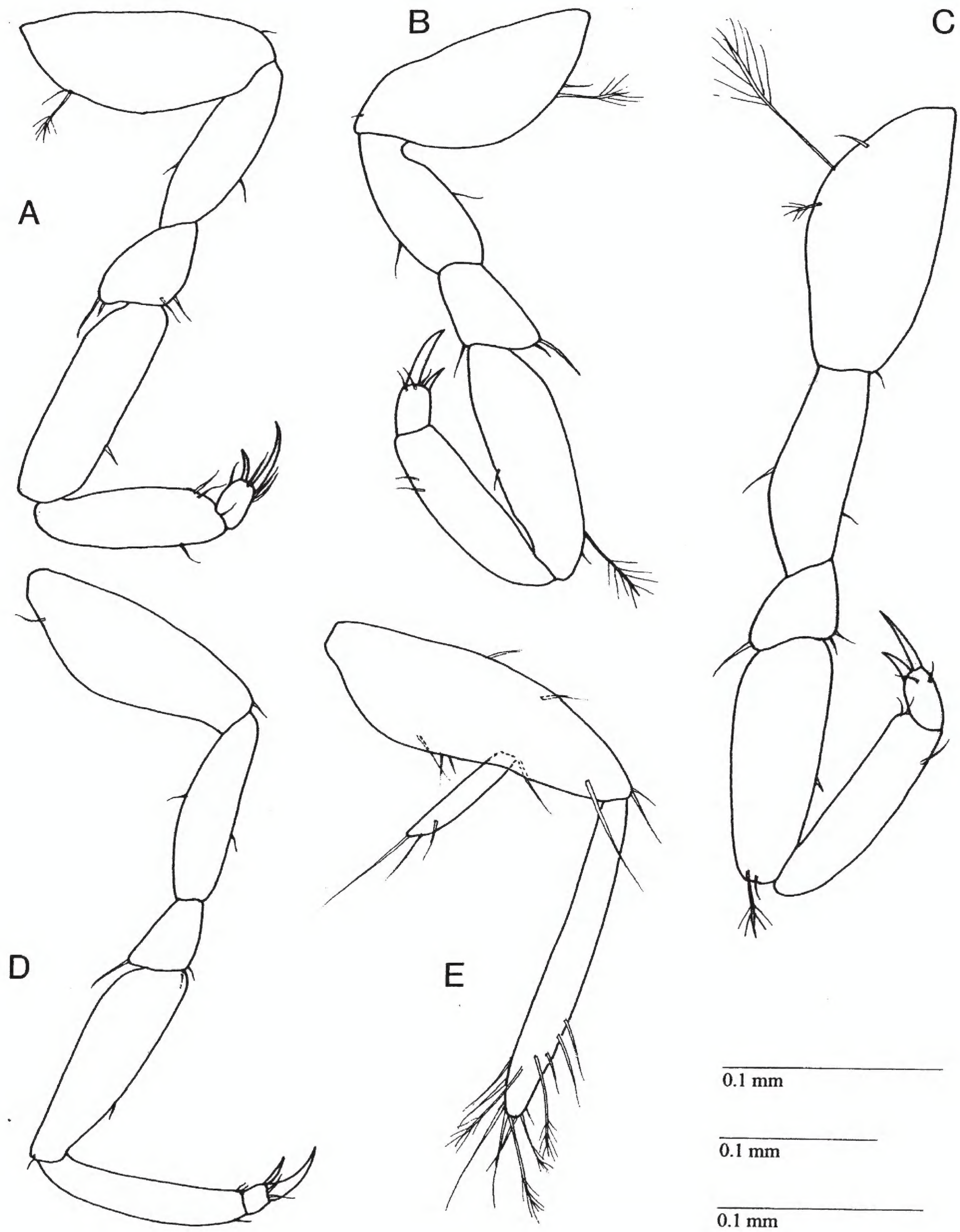

Fig. 3 - *Microcharon boulanouari* n. sp. A, pereiopod 1; B, pereiopod 3; C, pereiopod 4; D, pereiopod 7; E, uropod. Scale bars: top: P1 to P4; middle: P7; bottom: uropod.

(not present in *M. boulanouari* n. sp.). In addition, one of the pereiopod claws is clearly longer in the Pyrenean species.

Several characters, such as the thinner body shape, the longer pereiopodal claw, the detailed morphology of the male pleopod 1, the round and

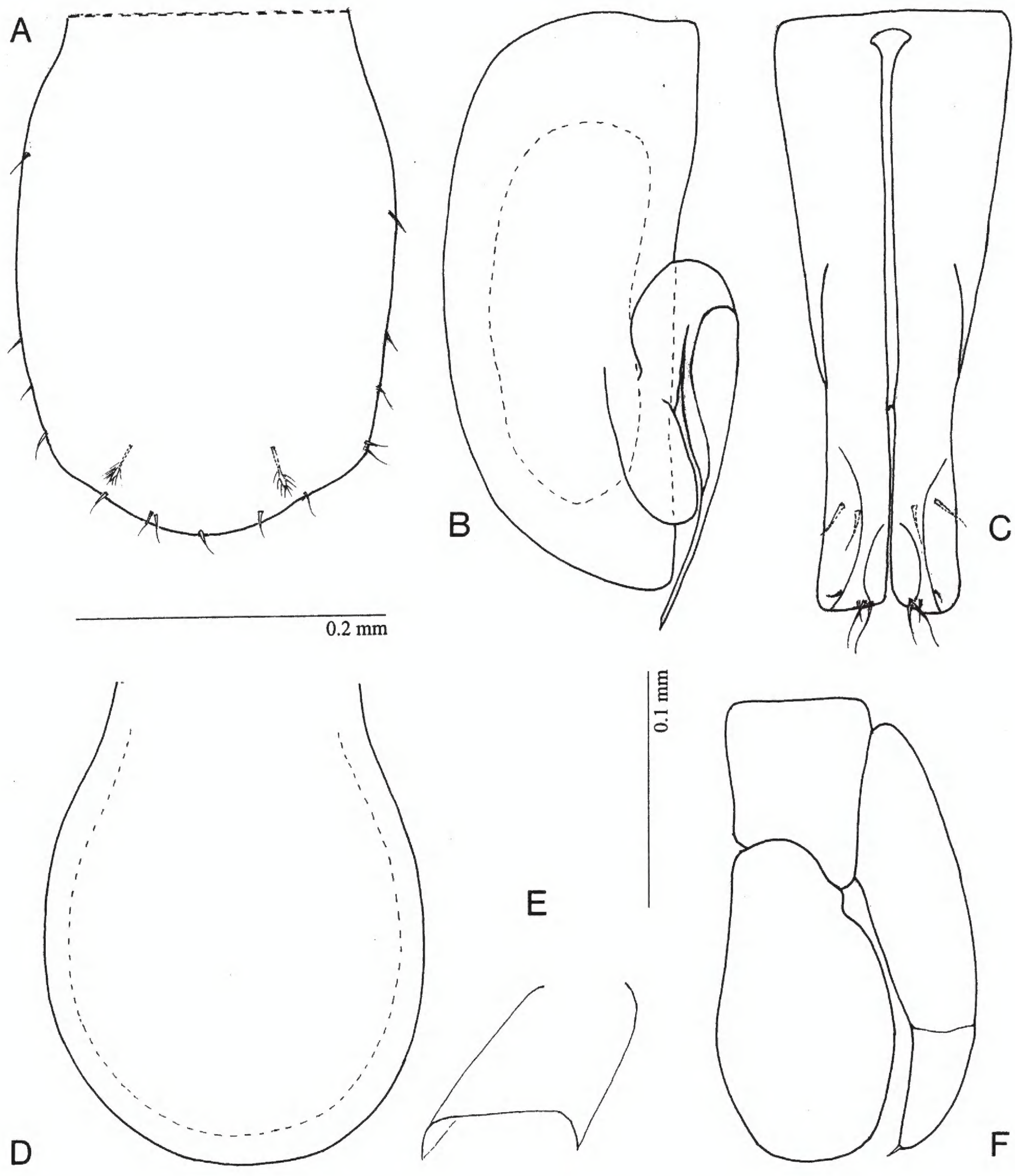

Fig. 4 - *Microcharon boulanouari* n. sp. A, pleotelson; B, male pleopod 2; C, male pleopod 1; D, female pleopod 2, ventral view; E, schematic 3D view of female pleopod 2; F, pleopod 3.

shorter female pleopod 2 are in an apomorphic state in *M. ariegensis* n. sp. and *M. rouchi*, in contrast to *M. boulanouari* n. sp.

Microcharon boulanouari n. sp. can be easily distinguished from the other French freshwater species, i. e. *M. angelieri*, *M. boui*, *M. juberthiei*, *M. doueti* and *M. reginae*, and from the Spanish Pyrenean species *M. longistylus* and *M. notenboomi* (Coineau 1968, Dole & Coineau 1987, Pesce & Galassi 1988a) by the following characters:

- length of longest plumose seta of second segment of antenna 1 slightly overreaching median part of segment 4 (versus seta longer in Spanish species and shorter in other French species);
- body segments relatively wide and stocky;

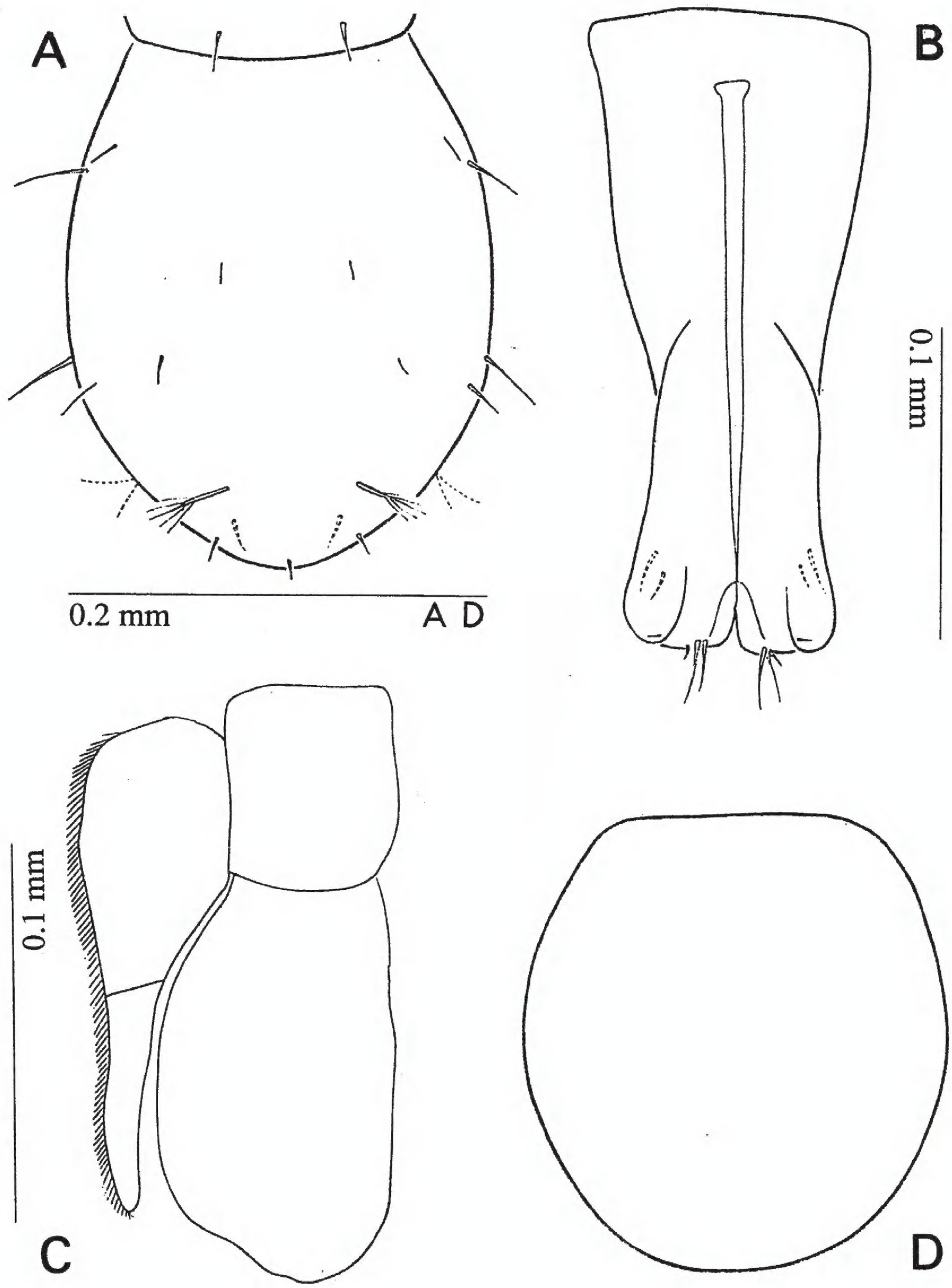

Fig. 5 - *Microcharon ariegensis* n. sp. A, pleotelson; B, male pleopod 2; C, pleopod 3; D, female pleopod 2.

- very long segment 5, and particularly 6 of antenna
- long pleotelson with characteristic distal margin;
- very long female pleopod 2 (vs more or less rounded appendage in other French species);
- sympod of male pleopod 2 not subovoid as in other French species, but with inner distal corner round and slightly extended.

Preliminary phylogenetic relationships

A phylogenetic study involving *Microcharon boulanouari* n. sp., *M. rouchi*, *M. ariegensis* n. sp., Moroccan, Algerian and Spanish species is in progress (Boulanour et al. in prep.). In the preliminary results of this study, and according to the phylogenetic relationships established within the freshwater species of *Microcharon* (Coineau 1994, Aït

Boughrous et al. 2007), *M. boulanouari* n. sp., *M. rouchi* and *M. ariegensis* n. sp. belong to the so-called "Western Mediterranean" group of species. More precisely, these three species share with members of this species group the most derived characters, i.e., female pleopod 2 without setae; uropods with the sympod shorter than the endopod; only three spines on the mandibular palp; and the inner distal corner of the sympod of male pleopod 2 not drawn out. The species *M. boulanouari* n. sp. is a sister species of the *rouchi-ariegensis* group, whereas *M. rouchi* and *M. ariegensis* n. sp. are two sister species.

Historical biogeography

Distributional pattern: *M. boulanouari* n. sp. occurs in the ground waters of the Aude River at Espéraza, Limoux, Carcassonne, Trèbes, from upstream to dowstream, and its Orbieu tributary at Ornaisons (Fig. 1). The Aude River flows within the western Languedoc in France. *M. ariegensis* n. sp. lives in the hyporheic waters of the Lachein, Hers and Ariège Rivers, in the northern Pyrenees, whereas *M. rouchi* inhabits the ground waters of the Nive, Laran and Kakouetta Rivers in the northern Pyrenean Basque Country.

All sites of the Aude basin inhabited by *Microcharon boulanouari* n. sp. are distributed in areas formerly covered by ancient marine gulfs. Upstream, the Aude River flows in the axial zone of the Pyrenees, while downstream, the floodplain is located within the former pre-Pyrenean trough excavated in the Senonian period (Bes 1988, Freytet 1973, Bousquet 1997) (Fig. 6). After the withdrawal of this Senonian Pyreneo-Provençal sea which was opened to the Atlantic Ocean (Debrand-Passard & Courbouleix 1984a, b, Plaziat 1981, 1983, 1984, Bilotte 1978, 1985), a new large Atlantic transgression developed towards the east up to Beziers within the north pre-Pyrenean trough, the Pyrenean domain, and its southern region in Spain during the Ilerdian (Late Paleocene-very early Eocene; Baceta et al 2005, Aguirre et al 2007, Gianetti 2010) (Fig. 7, 8). Almost the entire future Aude basin was covered by this Biscay embayment, as well as the Adour basin in the Aquitaine region (Tambareau 1972, Plaziat 1981, 1984, 1986, Alabouvette et al 1984, Bilotte et al 1989, Payros et al. 2006). The sea retreated definitively from the northern Pyrenean regions from the late Ilerdian to the Cuizian (= Early Eocene, 52-46 Myr (Odin & Odin 2002); Tambareau 1972, Tambareau et al 1997, Plaziat 1981, 1984, 1986, Freytet 1973). No further Atlantic transgression reached the Languedoc area of the Aude and the Ariège basins. The common ancestral populations of *Microcharon boulanouari* n. sp., *M. rouchi* and *M. ariegensis* n. sp. lived in the interstitial littoral water or in the shallow bottoms of the Ilerdian marine gulf. They settled in fresh ground waters during the regression of this Ilerdian embayment (Fig. 9) from the late Ilerdian onwards, according to the second stage of the Two Step Model of Colonization and Evolution. In the region of the central Pyrenees, the Ilerdian gulf retreated from the Ariège area during the Cuisian (46 Myr), slightly after from the Aude basin. *M. ariegensis* n. sp. ancestral populations settled in fresh subterranean waters in the very early Eocene, whereas *M. rouchi* ancestors were left in fresh ground waters much later, when the sea retreated from the Basque-Country in the Eocene-Early Oligocene (34-30 Myr). The Pyrenean uplift at the end of the middle Eocene (i.e. end of the Lutetian, 42 Myr, during the Biarritzean and the Early Priabonian periods) (Durand-Delga 1980, Arthaud & Seguret 1981, Baceta et al 2005, Bilotte et al 2007, Scheibner et al 2007) induced subsequent erosion phenomena resulting in the development of the hydrographic systems of the Aude River, the Hers and the Ariège Rivers, and the Adour River, in separate valleys. At the same time, the primitively continuous ancestral population was divided in several isolated populations which further evolved independently by vicariance processes in each river catchment. Such vicariance evolution resulted in the closely related species *M. boulanouari* n. sp. in the Aude River ground waters on the one hand, and the two Pyrenean species *M. rouchi* from the Nive and the Laran Rivers (western northern Pyrenees, the Basque Country), and *M. ariegensis* n. sp. from the Lachein and the Ariège basins (northern central Pyrenees) on the other hand. Moreover, the sea retreated much later in the Basque Country. Different extant species live today in the three different hydrographic basins and are endemic to each basin. They are rhoendemics sensu Myers & De Grave (2000).

DISCUSSION

One of our goals was to describe the new species *Microcharon boulanouari* and to compare it with other Spanish and French species of the genus in order to get reliable characters for phylogenetic analyses of several groups of species from the Mediterranean. *M. boulanouari* n. sp., from the Aude River ground waters, displays several characters in a plesiomorphic state whereas these characters are in an apomorphic condition in the two Pyrenean species *M. ariegensis* n. sp. and *M. rouchi* from the Ariège and the French Basque Country regions. These morphological differences may reflect the significant role played by the marine regression of the Ilerdian gulf on the speciation. It occurred by

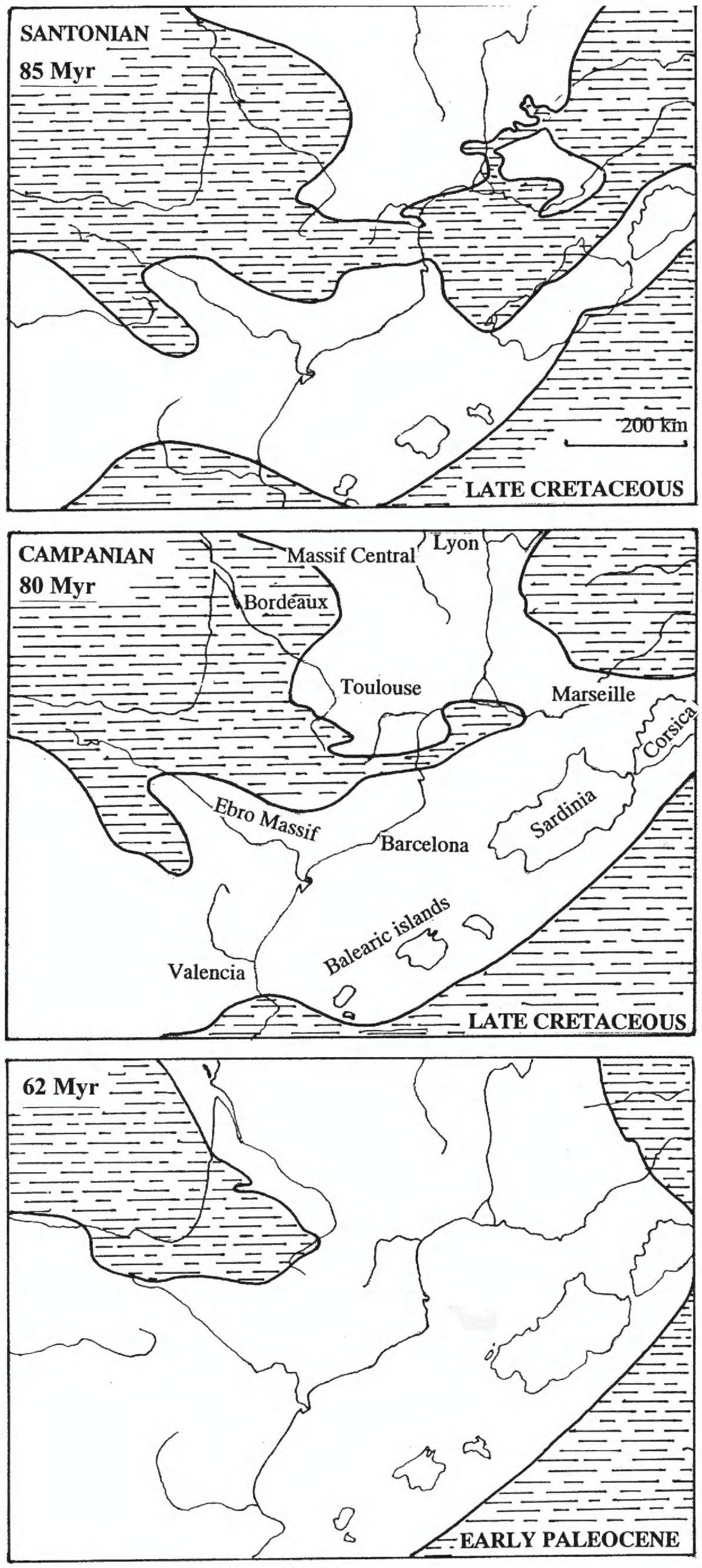

Fig. 6 - Southwestern France and northeastern Spain during the Santonian (top), the Campanian (middle) and the Early Paleocene (bottom): extension of the marine embayments (hatched areas). After Plaziat 1981 and Debrand-Passard & Couberleix 1984a, b, modified.

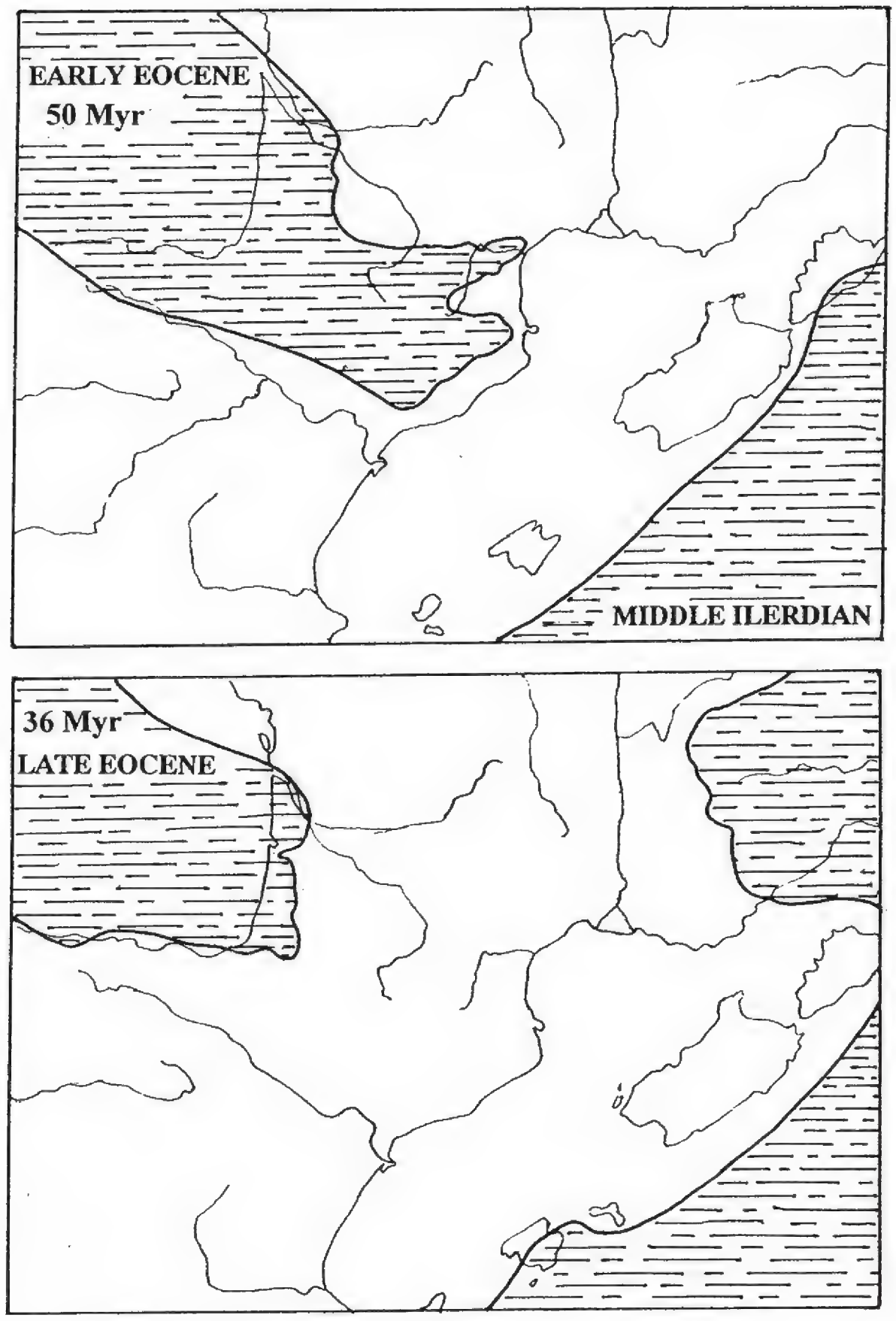

Fig. 7 - Soutwestern France and northeastern Spain during the Early Eocene (Ilerdian period, top) and the late Eocene (bottom): extension of the marine gulfs (hatched areas). After Plaziat, 1981 and Debrand-Passard & Courbouleix 1984a, b, modified.

successive stages from the East to the West (Plaziat 1981, 1986). The distributional pattern of these species, that are marine relicts, corresponds to the successive locations of the shoreline. The ancestors of *M. boulanouari* n. sp. stranded first into fresh ground waters while the ancestral populations of the Pyrenean species remained for a longer time in the unstable environment of the marine shallow bottoms or of the littoral interstitial water before their entrance into subsurface water-bodies later. Such a changing environment induced new selection pressures that may have triggered subsequent rapid evolutionary change. In contrast, at the same time the ancestors of *M. boulanouari* evolved in the more stable environment of the inland ground waters (Gibert et al. 1994). So that the extant species retains plesiomorphic character states. This species may have evolved at a lower rate. Similarly, various authors have reported that an increase of evolutionary rates is related to environmental instability and stress, from the molecular scale to that of biogeography (Parsons 1991, Taddei et al 1997, Stearns 2002, Boutin & Coineau 1991, 2000, Ruokolainen et al 2009, Venditti & Pagel 2010). Stearns & Hoekstra (2002) added that stasis is the "result of lack of change in the environment, and therefore in selection pressure". At the molecular scale, Wägele et al (2003) concluded also that the evolution of Asellota isopods is faster in marine than in freshwater habitats and that the low number of substitutions seen in sequences of freshwater species is caused by slow rates of adaptive morphological evolution.

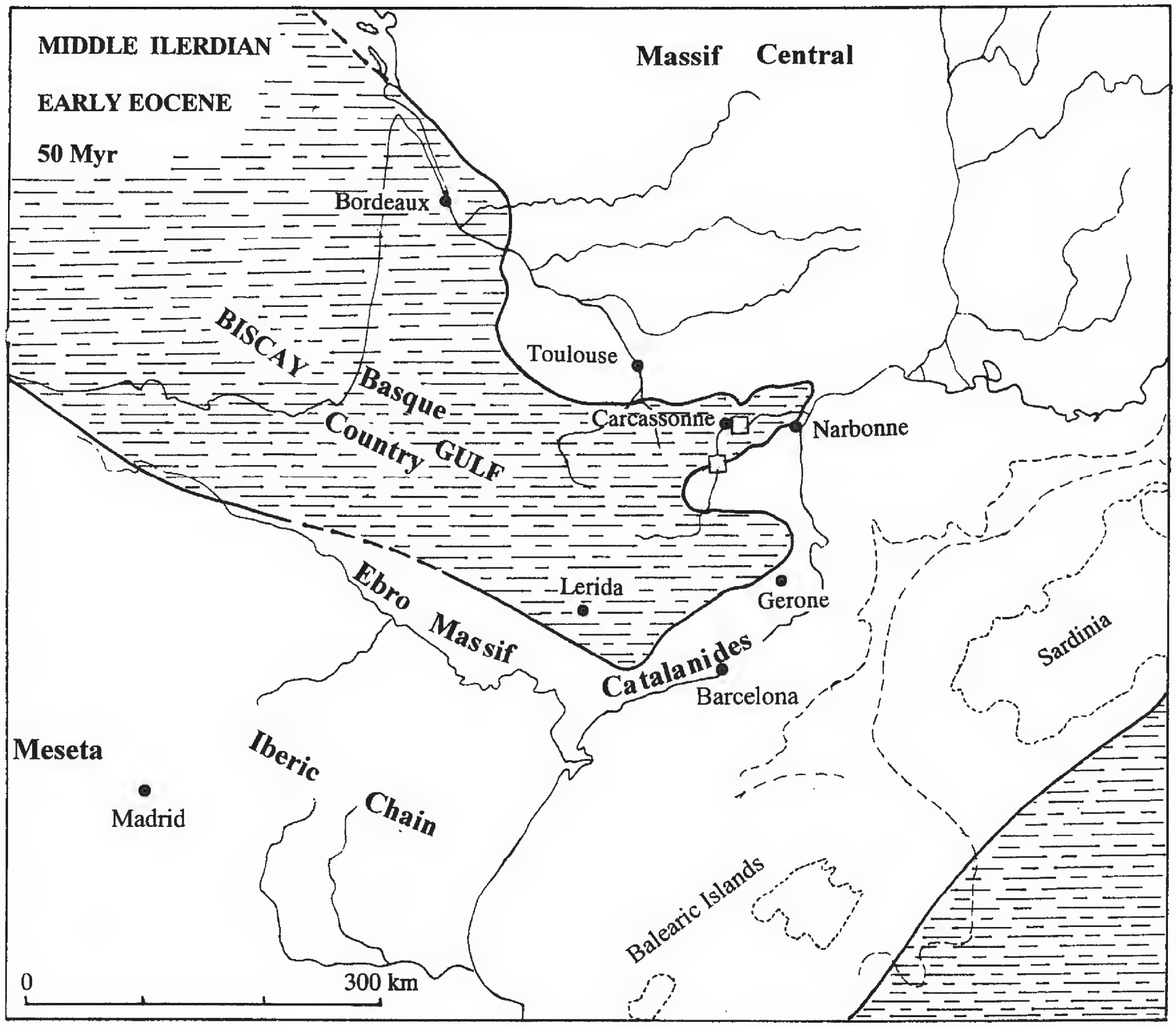

Fig. 8 - Southwestern France and northeastern Spain during the early Eocene period: extension of the marine Biscay gulf (hatched) and distribution of *Microcharon boulanouari* n. sp. (open squares). After Plaziat 1981 and Debrand-Passard & Courbouleix 1984a, b, modified.

Besides successive environmental changes, *Microcharon* diversification in southwestern France is also linked to the isolation of ancestral populations due to the Pyrenean orogenesis. Subsequent vicariant processes in the different new river catchments resulted in endemic species occurring in only one hydrographic system. A high level of endemism is one of the general patterns emerging in the outcome of the large-scale European survey of groundwater biodiversity in the framework of the European PASCALIS project (Gibert & Culver 2009, Gibert et al 2009, Deharveng et al 2009). Trontelj et al (2009) states that this pronounced endemism "is caused by strong hydrographical isolation and low dispersal abilities of their inhabitants". Small ranges of stygobionts are the rule and "ranges over 200 km are extremely rare". *Microcharon* species that are restricted to one hydrographic system, even to one tributary, may belong to the so-called "narrow or short-range endemics" (Eberhard et al 2009).

According to Trontelj et al (2009), stygobiont ranges seem to "reflect historical rather than current hydrogeological conditions". Therefore, geological history is an important determinant of species richness. The central Pyrenees are one of the four hotspots of groundwater biodiversity identified in Europe (Deharveng et al. 2009). Therefore, filling the gap of sampling coverage in the aquifers between the eastern and the western Pyrenees may give the opportunity to discover new species also derived from the Ilerdian marine gulf. Such species will allow to test sensu K Popper our palaeobiogeographic hypotheses.

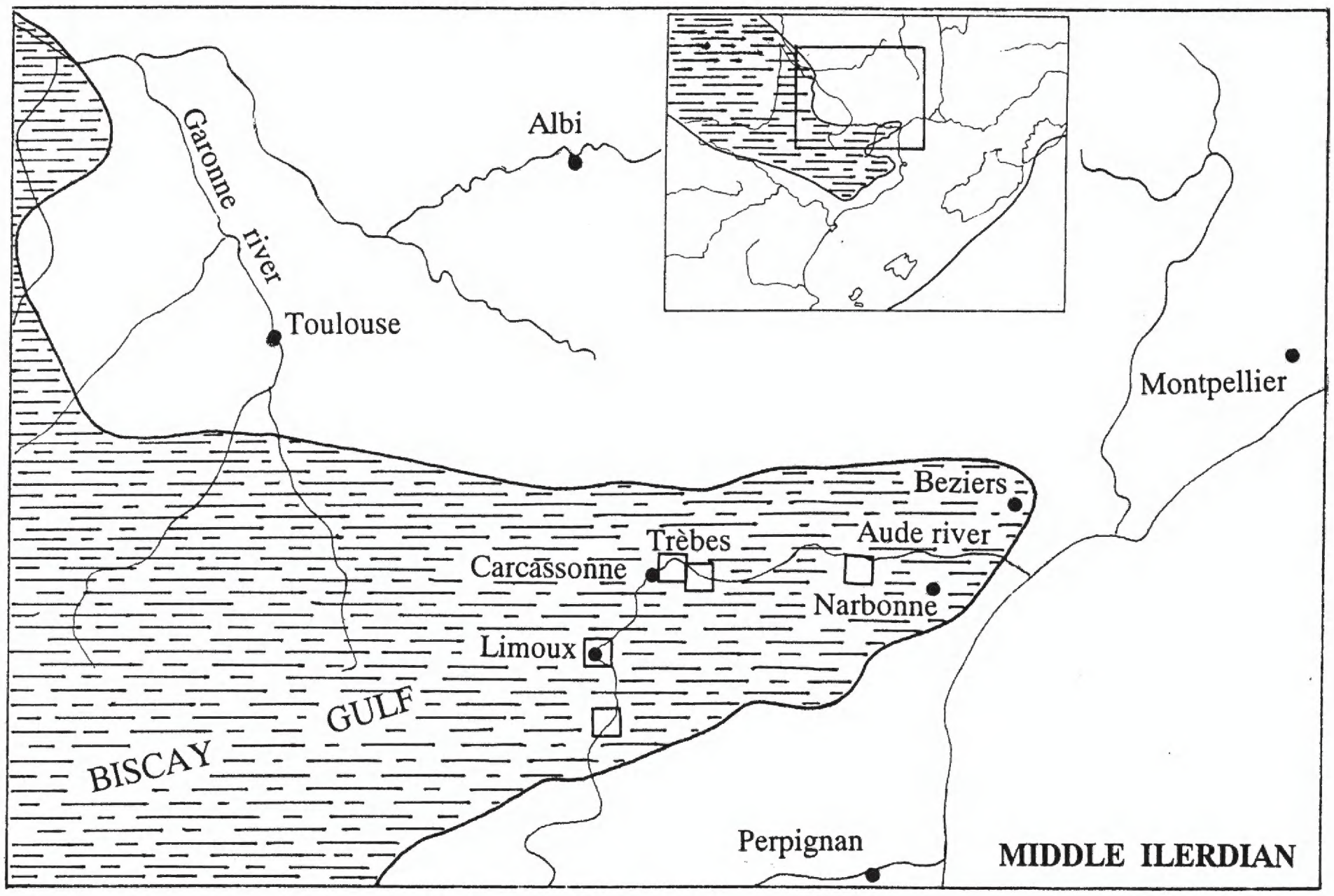

Fig. 9 - Extension of the Ilerdian embayment in the western Languedoc (hatched area) and location of *Microcharon boulanouari* n. sp. sites (open squares). After Plaziat 1981 and Debrand-Passard & Courbouleix 1984a, b, modified.

ACKNOWLEGMENTS

With the support of the European project PASCALIS (Protocols for the Assessment and Conservation of Aquatic Life in the Subsurface) EVK2-CT-2001-00121. Thanks are due to late J. Gibert, leader of the project PASCALIS and L. Deharveng, responsible for the ECOTOUL team, the universities Pierre et Marie Curie (Paris VI)-Observatoire Océanologique de Banyuls, Paul Sabatier (Toulouse III) and Cadi Ayyad (Marrakesh) for facilities. We also thank M Boulanouar for his collaboration. We are grateful to the anonymous reviewers for their comments.

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